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ORIGINAL ARTICLE

Monitoring and Assessing Health Risk of Exposure to Nitrate Residues in Agricultural Products; Case Study in Qom Province, Iran

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KEYWORDS

Nitrate;
Monitoring;
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ABSTRACT: This study was designed to investigate the rate of nitrate residual of major vegetables marketed in the region of Qom province and to estimate the human health risk assessment. In the first stage of this work, four groups of vegetables (lettuce, tomatoes, onion and potatoes) were selected. In two seasons, summer, and autumn, for 4 months165 vegetable samples (lettuce 35, tomatoes 45, onion 35, and potatoes 40) were analyzed to assess the levels of nitrates in vegetables. In the second stage of the study, health risk assessment from intake of vegetables was investigated. Concentration of nitrate in vegetables samples including lettuce (200 ± 65 mg kg^{-1}), onion (187 ± 43 mg kg^{-1}), potato (87 ± 33 mg kg^{-1}) and tomato (294 ± 67 mg kg^{-1}) were determined. Results showed that the average daily dose of nitrate (mg/kg/day) for age groups from vegetables distributed in Qom province is less than the ADI value (3.70 mg/kg BW/day) and the hazard quotient (HQ) of nitrate was less than 1, respectively, so that consumption of such vegetables is not dangerous to consumers' health. Hazard index (HI) for all age groups was less than one were calculated, however, the hazard index is higher for the age group of 5-18 years compared to other age groups. According the results of the cancer risk assessment, the mean cancer risk calculated for all groups by consumption of onion, tomato, and potato products is higher than the acceptable limits (the acceptable level of 1 case per 10000) estimated. Although the results obtained from the health risk assessment of nitrate residual amount in vegetable samples showed no significant hazard for consumers, due to the large share of vegetables in the daily food basket, it is inevitable to continuously monitor nitrate during cultivation and storing the product at the distribution levels.

INTRODUCTION

Nitrate is one of the important pollutants that accumulate mainly in plant organs because of unbalanced fertilizer application and excessive use of nitrogen fertilizers. Excess nitrate may contaminate surface and groundwater through leaching and soil erosion [1]. Distribution and Nitrate uptake in plants is of great importance due to environmental concerns and the quality of agricultural products. The chemical fertilizers used are known as one of the effective factors in nitrate accumulation. The accumulation of nitrate in vegetables often depends on the

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amount and type of nutrients in the soil and is closely related to the amount and timing of chemical fertilizers [2, 3].Due the adverse effects of nitrate on human health, great attention has been paid to the accumulation of this ion in vegetables. Accumulated nitrates in vegetables are converted into nitrites and nitrous of acids; if these substances are combined with the amines of the first and second types, nitrosamines are produced, the health hazards of which have been proven.

to maintain the health of food consumers and achieve food safety, monitoring of pollutants residual must be considered as an important factor. Complications of nitrate in humans occur as a result of its reduction by gastrointestinal bacteria and its conversion to nitrite. Nitrite formation, which leads to the oxidation of hemoglobin in the blood and its conversion to methemoglobin, that this process, oxygen delivery to the body's cells is disrupted [2]. This causes "methemoglobinemia" or "Blue Baby Syndrome" in newborn. The results of studies and the latest study in Iran show that food consumption, lettuce, tomatoes, and potatoes had the highest per capita consumption in the groups of leafy, shrubby, and tuberous vegetables. As the potato crop is one of the most important agricultural products, its per capita consumption is in the country after wheat and rice [5]. In recent years according to geographical location and climatic conditions, the provincial population of Qom has increased and most of the required agricultural products of Qom province are supplied from other provinces. Due to the need for residual monitoring of pesticides, heavy metals, and nitrates in agricultural products, as well as the lack of a continuous system for monitoring pesticides and other pollutants on these products and the lack of necessary studies in this field, this study was designed to investigate the rate of nitrate residual of major vegetables marketed in the region of Qom province and conduct of human health risk assessment.

MATERIALS AND METHODS

Setting of study

This cross-sectional study was designed and accomplished in two-stages in the Qom city with approximately 1300,000 habitants. In the first stage, four groups of vegetables (lettuce, tomatoes, onion, and potatoes) from the main Fruit and Vegetables Supply Center of Qom were collected (Figure1). Then, 165 vegetable samples (lettuce: 35, tomato: 45, onion: 35, and potato: 40) were analyzed to assess the levels of nitrates. The sampling time in this study is related to the summer and autumn months of 2021. The sample size in this study was calculated based on the results of study records in Iran and the amount of nitrate in similar products.

In the second stage, health risk assessment from the intake of vegetables was investigated.

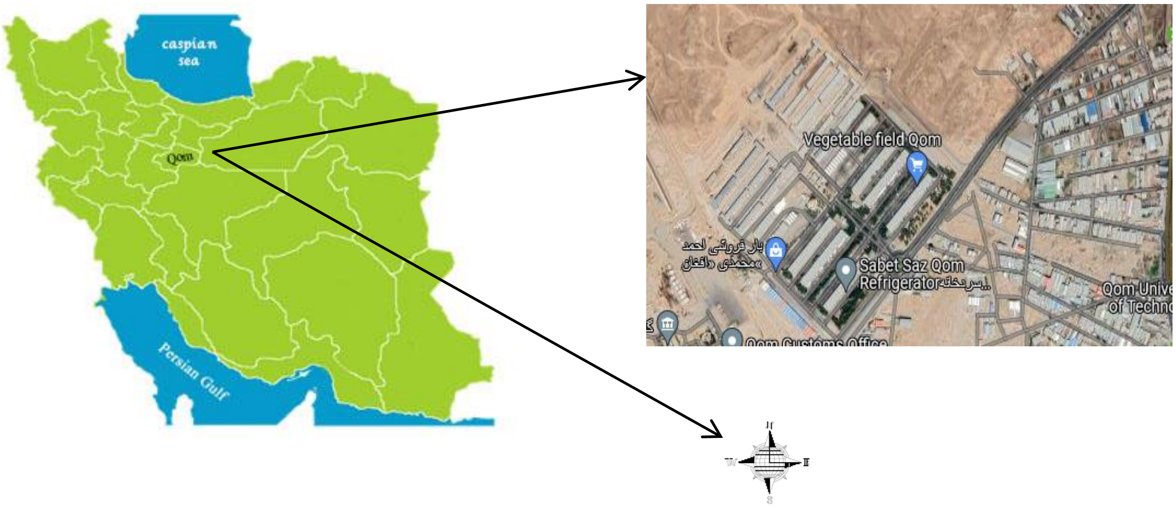

Figure 1. Location of the study area

Chemicals and reagents, Sample preparation, and Method validation

For each of the potato, tomato, onion, and lettuce products offered in Fruit and Vegetable Market in Qom in the two seasons of summer and winter, according to study records and using the following formula and by considering a confidence interval of 95% (Z_{1-a}), test accuracy of 5% (d), and P=54%, the sample size was estimated: n= $(Z_{1-a})^{2}$*P (1-P)/d^2 , 90 samples of potatoes, 80 samples of tomatoes and 40 samples of lettuce, including 45 sampling periods (Weight of each sample 1kg) were collected. Preparation of samples included separation of the non-edible pieces, washing, and freezing. To prepare the samples, first the non-edible parts are separated and after washing the samples, about 200±2 g of the sample is dried, crushed and homogenized and prepared by freezing dryer (drying process at 80 Ċ was performed for 24 hours). To prepare of stock solution (1000ppm), 1.6 g of potassium nitrate powder without water with deionized water is brought to a volume of 1 liter and the required working standards are prepared from it. The prepared samples are passed through a 40 mesh sieve after pulverization. Then weigh 0.1 g of the sample and add it to 10 ml of ion-free distilled water. The resulting suspension was kept in an oven at 45°C for one hour; the liquid was stirred and filtered through filter paper or a centrifuge (15 minutes at 5000 rpm). To each of the control and samples of the prepared product, 2 ml of sodium-salt saturated solution, 10 ml of sulfuric acid, and 0.5 ml of bruscin sulfanilic acid solution were added. After 20 minutes of heating in Ben Marie at a temperature above 95 and after cooling the absorption value was read at 410 nm by spectrophotometer. In this study the method fitted a linear model with (R2= 0.974) and limit of detection (LOD) 1 mg/l was calculated as (Figure 2) [6].

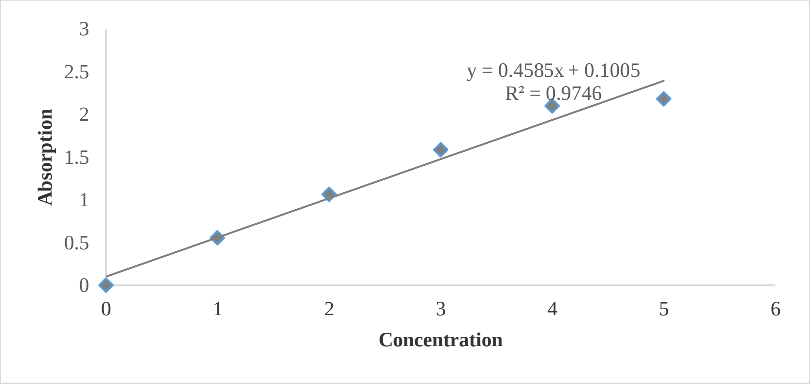

Figure 2. Calibration curve of Spectrophotometer- UV in 560 nm.

Health risk assessment

To conduct the health risk assessment, the acceptable daily intake (ADI) for the nitrate ion was 3.7 mg kg^{-1} of body weight according to the Joint FAO/WHO Expert Committee on Food Additives (Jecfa). The Average daily dose (ADD) was calculated according to the following equation 1

$$AAD = \frac{C \times IR \times ED \times EF}{BW \times AT} \quad (1)$$

C: represent the quantity of nitrate in vegetables (ppm)

ED: exposure duration about concerning the age range of population containing 5-18,19-29, 30-59, and > 60, respectively.

IR: vegetable consumption pattern (g/day) that in this study, per capita consumption of onions was 46 gr/day, lettuce 14 gr/day, tomatoes 42 gr/day, and potatoes 128 gr/day were considered. Also, Based on the data gathered from respondents, the frequencies of consumption in the household food basket: lettuce 2 times a month, onion 3 times a week, potato 3 times a week, and tomato 2 times a week were estimated.

EF: exposure frequency (365 days/year)

BW: body weight for age groups according to the weight of fifty percentile of the corresponding WHO age-weight curve, the age range of 5-18 (60 kg) and older as 70 kg was considered.

AT: average lifetime (ED×365 days/years)

The hazard quotient (HQ) for risk characterization was estimated through Eq.2:

$$HQ = \frac{ADD}{ADI} \qquad (2)$$

The interpretation of the hazard quotient contains: HQ <1.0 that indicated no significant risk and HQ >1.0 which indicated significant risk [10]. Hazard index (HI), which is obtained by summing the HQ values, was determined by Eq.3:

$$HI = \Sigma HQ \qquad (3)$$ [7].

Cancer risk assessment (CR) to nitrate by intake of vegetables, was calculated through (Eq.4).

$$CR = LADD \times CSF \quad (4)$$

LADD: lifetime average daily dose

CSF: cancer slope factor value of nitrate 1.04×10^{-2} (mg/kg BW/day) were determined [8, 9]. In this study for cancer risk, acceptable lifetime cancer risks are below 10^{-4}[10].

Data were gathered using the tools of the structured questionnaire (NUTRIKAP) and interviews with 1000 people who were responsible for food preparation for the family as well as a qualified person who was older than 15 years old [11]. The validity and reliability of the questionnaire were assessed and confirmed by nutritional experts in health centers. The sampling method at the household level was the single-stage cluster sampling (20 clusters of 50 people in Qom Province).

RESULTS

Quantity of nitrate concentration in the vegetables

The concentrations of nitrate in four groups of vegetable samples (Lettuce, Onion, Potato and Tomato) are presented in Table 1.

Table 1. Nitrate concentrations (mg kg^{-1}) in the examined samples

Samples characteristics	Lettuce	Onion	Potato	Tomato
Sample Size	35	40	45	45
Mean ± SD (concentration)	200 ± 65	185 ± 43	87 ± 33	294 ± 67
maximum residue level (MRI)	1500	90	170	120

Exposure and health risk assessment

In this study, according to the steps of a health risk assessment process, the results are shown in Tables (2, 3, and 4). Daily consumption of vegetables and bodyweight of age groups as described in Table 2.

The average daily dose and lifetime average daily dose of nitrate, it was calculated based on the values shown in Table 3.

Table 2. Estimated daily consumption of vegetables and bodyweight of age groups

Age Range	Lettuce		Onion		Potato		Tomato	
	IR	BW	IR	BW	IR	BW	IR	BW
5-18	35	29	46	29	100	29	31	29
19-29	45	70	46	70	120	70	41	70
30-59	45	70	46	70	120	70	41	70
>60	45	70	46	70	120	70	41	70

Table 3. Average daily dose and life time average daily dose of nitrate (mg/kg/day) for age groups

Age Range	Lettuce		Onion		Potato		Tomato	
	ADD	**LADD**	**ADD**	**LADD**	**ADD**	**LADD**	**ADD**	**LADD**
5-18	0.021	0.0036	0.180	0.030	0.16	0.028	0.1	0.017
19-29	0.0011	0.004	0.077	0.027	0.067	0.024	0.083	0.029
30-59	0.0011	0.00062	0.077	0.044	0.067	0.038	0.083	0.046
>60	0.0011	0.00094	0.077	0.070	0.067	0.071	0.083	0.075

Table 4. Hazard Quotient and cancer risk of nitrate for age groups from vegetable consumption in Qom province.

Age Range	Lettuce		Onion		Potato		Tomato		HI
	HQ	**CR**	**HQ**	**CR**	**HQ**	**CR**	**HQ**	**CR**	**(Σ HQ)**
5-18	0.0056	37E-6	0.048	312E-6	0.043	29E-5	0.027	17E-5	0.123
19-29	0.00029	41E-6	0.020	280E-6	0.018	24E-5	0.022	30E-5	0.06
30-59	0.00029	64E-7	0.020	457E-6	0.018	39E-5	0.022	47E-5	0.06
>60	0.00029	97E-7	0.020	72E-5	0.018	73E-5	0.022	78E-5	0.06

DISCUSSION

Concentration of nitrate in vegetables samples including lettuce (200 ± 65 $\mathrm{mg\,kg^{-1}}$), onion (187 ± 43 $\mathrm{mg\,kg^{-1}}$), potato (87 ± 33 $\mathrm{mg\,kg^{-1}}$) and tomato (294 ± 67 $\mathrm{mg\,kg^{-1}}$) were determined. According to the obtained results that were shown in Table 1, nitrate concentration in 58.57% of onion and 82.8% of tomato samples have higher than the limit recommended by the Institute of Standards and Industrial Research of Iran (ISIRI) while in other samples, including potato (87 ± 33 $\mathrm{mg\,kg^{-1}}$) and lettuce (200 ± 65 $\mathrm{mg\,kg^{-1}}$), were lower than the permissible limit [12].

In this study, the results of Tables 2, 3, and 4 demonstrated that the average daily dose of nitrate (mg/kg/day) for the age groups from vegetables distributed in Qom Province was less than the ADI value (3.70 mg/kg BW/day) and hazard quotient (HQ) of nitrate was less than 1. Therefore, the consumption of such vegetables is not dangerous for consumers' health. According to the consumption pattern of vegetables, the hazard index (HI) for all the age groups was less than one; however, the hazard index was higher for the age group of 5-18 years old than for other age groups. The intake of nitrate in Qom Province may be a concern for young age groups. In the cancer risk assessment with the results shown in Table 4, the mean cancer risk calculated for all the groups by consumption of onion, tomato,and potato products was higher than the estimated acceptable limits (the acceptable level of 1 case per 10000).

The results of the present study are consistent with the study of Mehri et al with aimed to assess nitrate concentration and its health risk in vegetable and fruit samples collected from Iran .results of Mehri et al was shown that the total hazard quotient (THQ) of nitrate was less than1 [13]. However, nitrate concentrations showed differences from the present study. In another study by Kiani et al related to Accumulation and human health risk assessment of nitrate in vegetables irrigated with different irrigation water sources- transfer evaluation of nitrate from soil to vegetables of Kermanshah in Iran, The highest amounts of nitrate were related to basil vegetables irrigated with well water and chemical fertilizer[14]. The results of Kiani et al showed that the average daily intake of nitrate through the consumption of vegetables grown in Kermanshah with any irrigation water is less than the allowable amount, so the consumption of such vegetables is not dangerous to consumers' health. In the study of Quijano et al, the exposure of the Valencia consumers to nitrate through the consumption of vegetable products appears to be relatively low [15]. The risk characterization indicates that, under the upper bound scenario, 0.79% of adults and 1.39% of young people can exceed the Acceptable Daily

Intake of nitrate. Overall, the estimated exposures to nitrate from vegetables are unlikely to result in noticeable health risks. The results of Quijano et al study have similarities with the present study. In general, by examining other studies conducted in Iran, it was found that the amount of nitrate can vary from 64.25 mg kg^{-1} (ppm) to 1024 mg /kg, which depends not only on genetic factors such as the type and species of plant product, but also under the influence of environmental factors such as the amount of soil nitrogen and the conditions of cultivation and storage [16, 17, 18]. A study of potential health risks of nitrate levels in fruits and vegetables by Uddin et al shows that root and tuber vegetables accumulate significantly higher levels of nitrate in comparison to fruits and fruit vegetables ($P < 0.05$)[7]. to better analyze the results of health risk assessment of nitrate in vegetables, it appears to be further studies would be useful based on epidemiological evidence and nitrate-nitrite conversion and methaemoglobin formation, in human food products to which nitrites have been added[19,20].

CONCLUSIONS

Although results obtained from health risk assessment of nitrate residual amount in vegetables samples show no significant hazard for consumer, due to the large share of vegetables in the daily food basket, continuous monitoring of nitrate and nitrite, during cultivation and storing of the product in the distribution levels is inevitable.

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Conflict of interests

Authors declares that he has no conflict of interest

REFERENCES

1. Council of Europe, Nitrates and Nitrites in Foodstuffs. Strasbourg, France, 1999.30 (pp. 29-36).

2. Corr`e W.J., Breimer T., 1979. Nitrate and nitrite in vegetables. Literature Survey, Department of Soils and Fertilizers Agricultural University Wageningen Netherlands. 3-5

3. European Food Safety Authority (EFSA), 2008. Nitrate in vegetables-Scientific Opinion of the Panel on Contaminants in the Food chain. EFSA Journal. 6(6), 689.

4. Larsson K., Darnerud P.O., Ilbäck N.G, Merino L., 2011. Estimated dietary intake of nitrite and nitrate in Swedish children. Food Additives and Contaminants. 28(5), 659-666.

5. Beheshti M., Shahbazi K., Bazargan K., Malekzadeh E., 2019. Study of Nitrate Status in Tomatoes and Cucumbers Distributed in the Alborz Province Market. Alborz University of Medical Sciences Journal. 8(3), 281-299

6. Institute of Standards and Industrial Research, 2014. Test and measurement of nitrite and nitrate in fruit and vegetable products by their measured molecular spectrum, Standard No. 4106.

7. Uddin R., Uddin M.T., Uddin M.A., Rabiul Islam G.M., 2021. Study of nitrate levels in fruits and vegetables to assess the potential health risks in Bangladesh. Scientific Reports. 11(1), 1-9.

8. Darvishmotevalli M., Moradniab M., Noorisepehr M., Fatehizadeh A., Fadaeid S., Mohammadi H., Salari M., Ali Jamali H., Daniali S.S., 2019. Evaluation of carcinogenic risks related to nitrate exposure in drinking water in Iran. Methods X .6, 1716-1727.

9. Kiani A., Sharafi K., Khalid Omerd A., Karami Matin B., Davoodi R., Sharafi H., Soleimani H., Massahi T., Ahmadi E., 2022. Accumulation and human health risk assessment of nitrate in vegetables irrigated with different irrigation water sources- transfer evaluation of nitrate from soil to vegetables, Environmental Research. 205, 112527.

10. Rahman M.M., Bodrud-Doza M., Muhib M.I., Hossain K.F., Sikder M.T., Shammi M., Akter R., Uddin M.K., 2020.Human health risk assessment of nitrate and trace metals via groundwater in Central Bangladesh. Pollution. 1; 6(2), 253-66.

11. Ahadi Z., Heshmat R., Sanaei M., Shafiee G., Ghaderpanahi M., Homami M.R., 2014. Knowledge, attitude and practice of urban and rural households towards

principles of nutrition in Iran: results of NUTRIKAP survey. J Diabetes Metab Disord. 13(1), 1.

12. Institute of Standards and Industrial Research, 2013. Maximum level for nitrate in agricultural products, Standard, ICS: 65.080.

13. Mehri F., Heshmati A., Moradi M., Mousavi Khaneghah A., 2019. The concentration and health risk assessment of nitrate in vegetables and fruits samples of Iran. Toxin Reviews. doi.org/10. 1080/15569543. 2019. 1673424

14. Kiani A., KiomarsSharafi., Khalid Omerd A., Karami Matin B., Davoodi R., Sharafi H., Soleimani H., Massahi T., Ahmadi E., 2022. Accumulation and human health risk assessment of nitrate in vegetables irrigated with different irrigation water sources- transfer evaluation of nitrate from soil to vegetables, Environmental Research. 205, 112527

15. Quijano L.,Vicent Y.F., McAllister C., Torres C., Pardo O., 2017.Risk assessment and monitoring programme of nitrates through vegetables in the Region of Valencia (Spain), Food and Chemical Toxicology. 100, 42-49

16. Yeganeh M., Bazargan K., Samaee M., Ardebili M.F., Tabbakhian S., 2019.Residual nitrate in potato tuber samples collected from fields in important production areas and the main fruit and vegetables supply center of Tehran. Iranian Journal of Soil Research. 32(4), 471-83.

17. Tabande L., Safarzadeh Shiraze S., 2018. Evaluation of nitrate accumulation and factors affecting it in some leafy vegetables in Zanjan Province. Iranian Journal of Soil Research. 23; 32(2), 189-201.

18. Iammarino M., Di Taranto A., Cristino M., 2014. Monitoring of nitrites and nitrates levels in leafy vegetables (spinach and lettuce): a contribution to risk assessment. J Sci Food Agric. 94, 773–778

19. Thomson B.M, Nokes C.J., Cressey P.J., 2007. Intake and risk assessment of nitrate and nitrite from New Zealand foods and drinking water. Food Additives & Contaminants. 24(2), 113-121.

20. Taneja P., Labhasetwar P., Nagarnaik P., Ensink J.H., 2017. The risk of cancer as a result of elevated levels of nitrate in drinking water and vegetables in Central India. Journal of Water and Health. 15(4), 602-14.